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Short Communication

Desensitization of Kitten Atria to Chronotropic, Inotropic and Adenylyl Cyclase Stimulating Effects of (−)Isoprenaline

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Summary. Desensitization of kitten atria with 30 μM (−)isoprenaline resulted in a 6-fold and 15-fold increase in the EC_{50}'s of (−)isoprenaline for its positive chronotropic effects (sinus pacemakers) and positive inotropic effects (left atria), respectively, but only in a 2-fold increase of the EC_{50} of (−)isoprenaline for adenylyl cyclase stimulation in membrane particles from atria. However, maximum cyclase stimulation by (−)isoprenaline was decreased to 1/2 in membranes from (−)isoprenaline-treated atria, whereas maximum increases in rate of sinus pacemakers and force of left atria were unchanged and reduced by 15%, respectively. The high affinity β-adrenoceptor blocker (−)bupranolol antagonized the adenylyl cyclase stimulation by (−)isoprenaline to similar extent in membranes from (−)isoprenaline and untreated atria, suggesting that the apparent affinity of β-adrenoceptors for ligands is unchanged by desensitization. The evidence is compatible with the concept that desensitization is associated with decreased availability of receptors and with the view that near maximal positive chronotropic effects of catecholamines may be caused by only threshold increases in membrane adenylyl cyclase activity.

Key words: (−)Isoprenaline desensitization – Kitten atria – Adenylyl cyclase – β-adrenoceptors – Sinus pacemaker and left atrial myocardium.

INTRODUCTION

Stimulation of adenylyl cyclase by catecholamines can be decreased by exposure of tissues or cells of various systems to high concentrations of amine (Deguchi and Axelrod, 1973; Franklin and Foster, 1973; Franklin et al., 1975; Mukherjee et al., 1975; Mickey et al., 1975; Hopkins, 1975). In heart, apparent affinities of many ligands for β-adrenoceptors mediating positive chronotropic and inotropic, relaxant and adenylyl cyclase stimulating effects of catecholamines are of the same order of magnitude, suggesting that a single receptor is involved in conveying heterogeneous effects (Kaumann and Birnbaumer, 1973, 1974b; Kaumann, 1974). However, 2 or 3 orders of magnitude greater concentrations of catecholamine are required to stimulate adenylyl cyclase in heart membrane particles (Kaumann and Birnbaumer, 1974a, b) than to cause positive chronotropic and inotropic effects (Kaumann, 1972). This dissociation suggests that stimulation of the adenylyl cyclase is either unrelated to the positive chronotropic and inotropic, and relaxant effects of catecholamines, or that small increases of cAMP modify substantially heart functions. The present experiments were carried out to study the relationship between the enhanced (−)isoprenaline-mediated production of cAMP by membrane bound adenylyl cyclase and the effects of (−)isoprenaline on sinus pacemakers and left atrial myocardium by comparing the desensitizations to each of these effects of the catecholamine.

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METHODS

Eight kittens of either sex, coming from 2 litters of 4 each, weighing 300 g (not weaned) were used. To reduce endogenous noradrenaline which may occupy β-adrenoceptors of membrane particles and act additively with exogenous catecholamine (Kaumann and Birnbaumer, 1974b, p. 7878), the kittens were pretreated with 2.5 mg/kg reserpine i.p. 18 h before sacrificing. The kittens were anaesthetized with chloroform; their hearts were washed free of blood with salt solution and rapidly removed. The physiological salt solution contained: (mM) Na^+ 140, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 1, Cl^- 98.5, SO_4^{2-} 1, HCO_3^- 29, HPO_4^{2-}, glucose 10, acetate 20, EDTA (ethylenediaminetetraacetic acid, disodium salt) 0.04. Water was redistilled

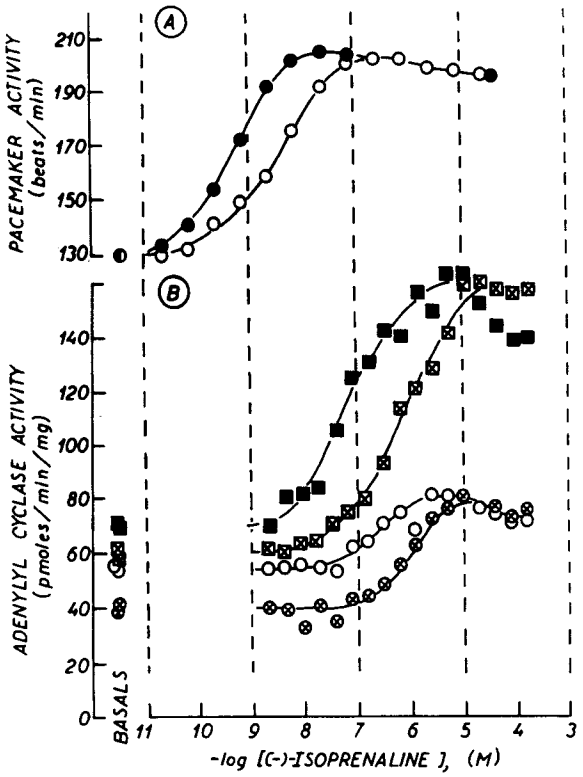

Fig. 1A and B. Desensitization at 32.5° C of a sinus pacemaker and atrial membranes to the chronotropic and adenylyl cyclase stimulating effects of (−)isoprenaline. (A) Spontaneously beating kitten atrium. Closed and open circles, concentration-effect curves for (−)isoprenaline before and 4 h after a 3-h exposure to 30 μM (−)isoprenaline. (B) Membrane particles of atria were incubated for 10 min in 50 μl of medium containing 1.9 mM [α-^{32}P]ATP (specific activity: 63 cpm/pmole), 3.5 mM $MgCl_2$, 1 mM EGTA, 1 mM [^{3}H]cAMP (8000 cpm), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 25 mM Tris-HCl pH 7.5, indicated concentrations of (−)isoprenaline without (black squares, open circles) and with (crossed symbols) 5 nM (−)bupranolol. The reaction was stopped by addition of a solution containing 10 mM cAMP, 40 mM ATP and 1% sodium dodecyl sulphate, followed by immediate boiling for 3.5 min. Membranes indicated by circles (17 μg protein assay) and squares (15 μg protein/assay) were from the atrium shown in (A) and from an electrically stimulated control atrium (not exposed to (−)isoprenaline), respectively

in glass. The solution was equilibrated with 5% CO_2 in O_2. The hearts were dissected in freshly oxygenated solution at room temperature. The muscles were set up at 32.5° C in an apparatus with a 50 ml bath described by Blinks (1965).

Four whole (to yield enough membrane particles) spontaneously beating right atria of the same litter were set up in pairs in 2 organ baths at a resting tension just sufficient for measurable development of tension. After an equilibrium period of 2 h, a cumulative concentration-effect curve for (−)isoprenaline up to 30 μM was determined on 2 of the atria. This latter concentration was left in contact with the tissues for 3 h. To mimic the influence of high beating rates on membrane adenylyl cyclase activity, the other 2 atria were driven at rates equivalent to the increases in rate by the various concentrations of (−)isoprenaline in the other 2 atria, and left at a rate of 200 min for 3 h, which was the average maximum rate of the 2 atria with (−)isoprenaline. After this period, (−)isoprenaline was washed out from 2 atria and electrical stimulation stopped in the other 2 atria. All 4 atria were washed repeatedly with physiological salt solution over a period of 4 h, until the rate of the 2 atria pretreated with (−)isoprenaline was stable. A second concentration-effect curve for (−)isoprenaline up to 20 μM was then determined on the (−)isoprenaline-pretreated atria while the other 2 atria were again driven as before. The drug was then washed away from the tissues for 15 min; stimulation of the other 2 atria was stopped and they were also washed. Membrane particles of both (−)isoprenaline-treated and untreated (electrically stimulated) atria were then prepared as described previously (Kaumann and Birnbaumer, 1974b) and stored at −70° C. Incubations of membrane particles for determination of adenylyl cyclase activities were carried out as detailed in Figure 1 and described by Kaumann and Birnbaumer (1974b). The [^{32}P]-cAMP formed and [^{3}H]-cAMP (added to incubations as a recovery marker) were isolated by the method of Salomon et al. (1974) and determined by liquid scintillation counting. The procedure of Lowry et al. (1951) was used for protein determination.

Four atria of the other litter were dissected into spontaneously beating right atria and left atrial strips. A right atrium and a left atrial strip were mounted in the same bath. The left atria were driven 30/min with square pulses of 5 ms duration applied through a punctate platinum cathode. Their resting tension was adjusted to 1/2 the level associated with maximum developed tension. Two successive concentration-effect curves for (−)isoprenaline were determined on these tissues before and after a 3-h exposure to 30 μM (−)isoprenaline, as described for the other 2 whole atria. Peak tensions of left atrial strips and rates of spontaneously beating atria were recorded with Statham transducers on an oscillograph.

RESULTS AND DISCUSSION

Exposure of atria to 30 μM (−)isoprenaline caused desensitization (Fig. 1, Table 1) to the amine with the following characteristics: The EC_{50}'s (−log M) of (−)isoprenaline increased from (mean ± S.E.M.) 9.17 ± 0.21 and 8.83 ± 0.20 to 8.39 ± 0.19 and 7.67 ± 0.21 in right (n = 6) and left (n = 4) atria, respectively. Maximum rates and force (mean ± S.E.M.) achieved with (−)isoprenaline were 190 ± 4 and 188 ± 3 beats/min in right atria and 2.8 ± 0.2 and 2.3 ± 0.2 g tension in left atria, before and 4 h after the 3-h incubation with 30 μM (−)isoprenaline. Equilibrium chronotropic effects of 30 μM (−)isoprenaline were somewhat smaller than maximum effects, and similar in undesensitized and desensitized atria (Fig. 1).

In membrane particles from the (−)isoprenaline-treated atria, maximal stimulation of adenylyl cyclase activity was only 1/2 of that of electrically stimulated atria (Fig. 1, Table 1). The EC_{50} (Fig. 1, Table 1) for cyclase stimulation by (−)isoprenaline increased somewhat from 88 nM to 167 nM. To see whether this small increase in EC_{50} was due to a decrease in apparent affinity of the β-adrenoceptors for their ligands, the potency of the high affinity, pure β-adrenoceptor blocker 1-(6′-chloro-3′-methylphenoxy)-tert.-(butylaminopropane-2-ol) [(−)KL255, (−)bupranolol] was investigated (Kaumann, 1972; Kaumann and

Table 1. Decrease of (−)isoprenaline stimulation of the adenylyl cyclase of membrane particles prepared from atria subjected to a 3-h treatment with 30 μM (−)isoprenaline

	Atrium	Basal[a, b]		(−)Isoprenaline				Stimulation due to (−)isoprenaline[e] (% over basal)
		none	5 nM (−)bupranolol	max.[a]	EC_{50} (μM)	5 nM (−)bupranolol		
						max.[a]	EC_{50} (μM)	
Control atria:								
Driven for 3 h at	1[c]	(70, 69)	(57, 61)	160	0.08	160	0.78	171
200 beats per min	2	(75, 76)	(51, 50)	148	0.10	144	0.49	185
Desensitized atria:								
30 μM (−)iso-	3	(85, 78)	(52, 57)	115	0.12	98	1.00	80
prenaline for 3 h	4[d]	(55, 59)	(40, 41)	82	0.22	81	1.06	100

[a] Activity in pmoles cAMP/min/mg protein.
[b] Values between brackets are duplicates.
[c] Data from the atrium represented with squares in Figure 1.
[d] Data from the atrium represented with circles in Figure 1.
[e] Calculated from activities obtained in the presence of 5 nM (−)bupranolol.

Birnbaumer, 1974b; Kaumann and Wittmann, 1975). Surprisingly, 5 nM (−)bupranolol decreased slightly, but significantly and to the same extent (Fig. 1, Table 1), basal cyclase activities in both membranes from (−)isoprenaline-treated and membranes from untreated (but electrically stimulated) atria, suggesting that some residual noradrenaline (not completely depleted by reserpine-pretreatment) was increasing cyclase activity and being antagonized by the blocker at the β-adrenoceptors. 5 nM (−)bupranolol antagonized the effect of (−)isoprenaline to similar extent in membranes from both (−)isoprenaline-treated and untreated atria. Thus, mean EC_{50}-ratios of (−)isoprenaline in the absence and presence of 5 nM (−)bupranolol were 7 and 6 for membranes of undesensitized and desensitized atria, respectively (Table 1).

The marginal increase of the EC_{50} for adenylyl cyclase stimulation by (−)isoprenaline and the similar antagonism by (−)bupranolol of the (−)isoprenaline effects in membranes from undesensitized and desensitized atria suggest that desensitization does not modify the affinity of the myocardial β-adrenoceptors for ligands. Basal adenylyl cyclase activities (obtained in the presence of 5 nM (−)bupranolol in membranes from untreated and (−)isoprenaline-treated atria were similar. This indicates that desensitization is not related to decreased enzyme activity.

Decreased maximal stimulation of adenylyl cyclase by (−)isoprenaline has been observed in desensitized frog erythrocytes; it was correlated with decreased binding but unchanged affinity of an adrenergic ligand, indicating that the number of available β-adrenoceptors was decreased (Murkherjee et al., 1975; Mickey et al., 1975). It is likely that the decrease of maximum stimulation of adenylyl cyclase by (−)isoprenaline in atrial membranes after desensitization is also related to a decrease of available receptors.

Because maximum adenylyl cyclase stimulation is decreased after desensitization, greater amounts of (−)isoprenaline are required to cause equivalent increases in absolute adenylyl cyclase activity after than before desensitization, in spite of persistence of unchanged affinity of β-adrenoceptors for adrenergic ligands. It may be therefore, that desensitization of adenylyl cyclase is the biochemical basis for the desensitization of chronotropic and inotropic effects of (−)isoprenaline, provided that desensitization does not alter the quantitative relationship between myocardial adenylyl cyclase activity and myocardial contractile events and pacemaker activity.

Maximum chronotropic effects of (−)isoprenaline were associated with only very small increases of adenylyl cyclase activity in membranes from both undesensitized and desensitized atria, indicating that only a small fraction of the possible cAMP producing power is sufficient to account for the chronotropic effects of (−)isoprenaline. Since maximum adenylyl cyclase stimulation is decreased in membranes from desensitized atria, a higher concentration of (−)isoprenaline is required in membranes from desensitized than from undesensitized atria to cause the same small increase of membrane adenylyl cyclase activity associated with maximum chronotropic effects of (−)isoprenaline. This phenomenon may at least partially account for desensitization of atria to (−)isoprenaline,

provided that cAMP production is related to positive chronotropic effects of (−)isoprenaline.

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